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Applicant ST. GEORGE'S ENTERPRISES LIMITED et al	

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<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
13 Dec 2002 (13.12.2002)	0229151.6	GB	05 Febr 2004 (05.02.2004)

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## PRIORITY DOCUMENT

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I also certify that the attached copy of the request for grant of a Patent (Form 1/77) bears an amendment, effected by this office, following a request by the applicant and agreed to by the Comptroller-General.

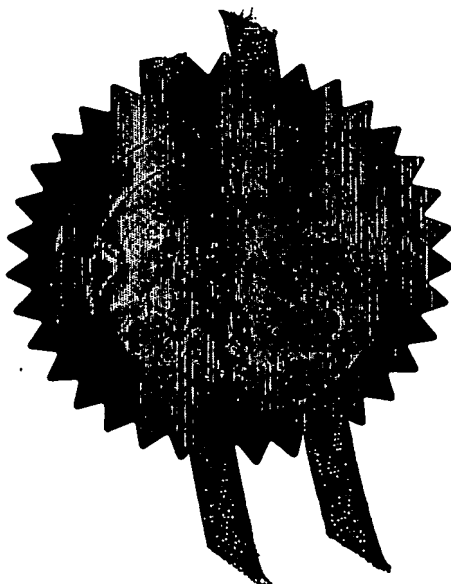
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13 DEC 2002

The Patent Office  
Cardiff Road  
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1. Your reference

★ 13 DEC 2002 12.1.78 185

2. Patent application number  
(The Patent Office will fill in this part)

0229151.6

3. Full name, address and postcode of the  
or of each applicant (underline all surnames)

St. George's Enterprises Ltd.  
Cranmer Terrace  
Tooting  
London  
SW17 0RE

Patents ADP number (if you know it)

7268311001

16DEC02 E771001-2 000027  
P01/7700 0.00-0229151.6

If the applicant is a corporate body, give  
country/state of incorporation

United Kingdom

4. Title of the invention

Product and Method

5. Name of your agent (if you have one)

~~Frank B. Dehn & Co.~~

JA KEMP

"Address for service" in the United Kingdom  
to which all correspondence should be sent  
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~~179 Queen Victoria Street~~  
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Patents ADP number (if you know it)

~~166001~~

6. If you are declaring priority from one or more  
earlier patent applications, give the country  
and the date of filing of the or of each of these  
earlier applications and (if you know it) the or  
each application number

Country

Priority application number  
(if you know it)

Date of filing  
(day / month / year)

7. If this application is divided or otherwise  
derived from an earlier UK application,  
give the number and the filing date of  
the earlier application

Number of earlier application

Date of filing  
(day / month / year)

8. Is a statement of inventorship and of right  
to grant of a patent required in support of  
this request? (Answer 'Yes' if:

yes

- a) any applicant named in part 3 is not an inventor, or
  - b) there is an inventor who is not named as an applicant, or
  - c) any named applicant is a corporate body.
- See note (d))

# Patents Form 1/77

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Description 35

Claim(s) 0 DNL

Abstract 0

Drawing(s) 8 + 8

10. If you are also filing any of the following, state how many against each item.

Priority documents 0

Translations of priority documents 0

Statement of inventorship and right to grant of a patent (Patents Form 7/77) 0

Request for preliminary examination and search (Patents Form 9/77) 0

Request for substantive examination (Patents Form 10/77) 0

Any other documents (please specify) 0

11. I/We request the grant of a patent on the basis of this application.

Signature

Date 13 December 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Elizabeth Jones  
020 7206 0600

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Product and Method

5       The present invention relates to molecules which  
impair PBX-dependent transcription regulation,  
particularly peptides which affect the binding of HOX to  
PBX and their use in a number of applications, including  
10       the reduction of aberrant cell division, e.g. to treat  
certain cancers, and to maintain pluripotency of stem  
cells, e.g. to maintain the pluripotency of stem cells  
for example during culture expansion.

      A variety of transcription factors are involved in  
the regulation of expression of proteins during  
15       embryogenesis and adult stem cell maturation. Homeobox  
(HOX) genes contain a highly conserved nucleotide  
sequence of about 180bp which encodes a homeodomain of  
about 60 amino acids. A homeodomain is a DNA-binding  
protein domain which can bind to target sequences in  
20       other genes and regulate their expression during  
development. The clustered Hox genes are key  
developmental regulators and are highly conserved  
throughout evolution. The homeotic HOX proteins which  
they encode share the 60 amino acid homeodomain and  
25       function as transcription factors to control axial  
patterning by regulating the transcription of  
subordinate downstream genes, e.g. developmental genes.

      In *Drosophila* and other insects there are eight  
different Hox genes that are encoded in two gene  
30       complexes, while in vertebrates there are 39 genes  
organized in four complexes. The four gene complexes  
are assigned the letters A to D. Based on sequence  
similarities the genes can be sorted into 13 "paralog"  
groups. The order of the paralogs along the chromosomes  
35       are conserved in the four complexes. The gene name is  
obtained by concatenating the gene complex letter  
designation with the group number, e.g. HOXA1, HOXB4

etc.

Pre-B-cell transformation related gene (PBX) is also an important regulatory protein that controls gene expression during development by interacting

5 cooperatively with HOX to bind to the target DNA (Mann et al., 1996, Trends Genet., 12(7), p258-262).

"Engrailed" proteins are also able to bind to PBX. PBX and HOX are known to interact via the hepta- or hexapeptide region on the HOX molecule, which is highly  
10 conserved (Phelan et al., 1995, Mol. Cell. Biol., 15(8), p3989-3997 and Neuteboom et al., 1995, PNAS, 92, p9166-9170). The hexapeptide is separated by a linker region from the N-terminus of the homeodomain. Once PBX and HOX have bound to one another, they enter the nucleus of  
15 a cell and there bind to target DNA and repress or activate that target gene's transcription.

Whilst these proteins are known to be involved in embryogenesis their precise roles have not been elucidated. Over or under expression of HOX proteins  
20 gives rise to a variety of consequences *in vitro* which implicate the involvement of these proteins in the control of differentiation processes. However the consequences of perturbing the interaction between PBX and the co-factors to which it binds have not been  
25 examined. Furthermore, PBX:HOX binding antagonists have been found to be rather specific to specific forms of the protein binding partners involved (see Peltenburg & Murre, 1996, EMBO Journal, 15(13), p.3385-3393). Furthermore, it is believed that the linker region  
30 between the homeobox and the hexapeptide is required for cooperative binding between PBX and HOX (Peltenburg & Murre, *supra* and Neuteboom et al., 1995, PNAS, 92, p9166-9170).

Surprisingly however, the present inventors have  
35 now developed a peptide which mimics the region of HOX to which PBX binds and acts as an antagonist of that binding. This peptide is based on the hexapeptide

region of HOXB-4 but has been found to have cross-reactivity (see Example 2) and reduces the binding of PBX to all HOX proteins. This is the first report of a peptide having such global PBX:HOX effects.

5 Furthermore, unlike other HOX-interacting peptides, the peptide of the present invention does not contain a linker region.

Surprisingly it has also been found that inhibiting the binding of PBX to its binding partners has profound and useful effects on stem cells, which allows the pluripotency of these cells to be maintained. It has also been found that as a converse effect, aberrant cell growth may be reduced to prevent or treat disorders or conditions in which such cell growth occurs. These findings offer significant clinical applications in which desired cells are protected and possibly expanded whilst the growth of detrimental cells may be prevented.

Thus, molecules which impair PBX-dependent transcription regulation (e.g. activation or repression), e.g. by interfering with the interaction between PBX and its co-factors, preferably HOX, and its target DNA, e.g. molecules which affect the binding of HOX and PBX proteins, have downstream effects which offer great advantages such as preventing or reducing aberrant cell division and maintaining pluripotency of stem cells.

In a first aspect therefore, the present invention provides a peptide having the following sequence:

WYPWMKKHH (Sequence (1))

30 which is preferably attached to a further peptide to form:

WYPWMKKHHRQIKIWFQNRMMKWKK (Sequence (2)),

or functionally equivalent derivatives, variants or fragments thereof.

35 "Functionally equivalent" derivatives, variants or fragments thereof refers to peptides related to, or derived from the amino acid sequence of Sequence (1)

(optionally with Sequence (2) attached), where the amino acid sequence has been modified by for example the use of modified amino acids or by single or multiple amino acid (e.g. at 1 to 10, e.g. 1 to 5, preferably 1 or 2 residues) substitution, addition and/or deletion but which nonetheless retain functional activity, insofar as they act as HOX mimics and thus antagonize the interaction between HOX proteins and PBX proteins (preferably PBX1 or PBX2) according to the assay described hereinafter (see Example 2, in which cross-linking of large molecules indicative of HOX:PBX binding is not observed).

Within the meaning of "addition" variants are included amino and/or carboxyl terminal fusion proteins or polypeptides, comprising an additional protein or polypeptide fused to the peptide sequence.

"Substitution" variants preferably involve the replacement of one or more amino acids with the same number of amino acids and making conservative substitutions.

Preferred "derivatives" or "variants" include those in which instead of the naturally occurring amino acid the amino acid which appears in the sequence is a structural analog thereof. Amino acids used in the sequences may also be derivatized or modified, e.g. labelled, providing the function of the peptide is not significantly adversely affected.

Derivatives and variants as described above may be prepared during synthesis of the peptide or by post-production modification, or when the peptide is in recombinant form using the known techniques of site-directed mutagenesis, random mutagenesis, or enzymatic cleavage and/or ligation of nucleic acids.

Functionally-equivalent "fragments" according to the invention may be made by truncation, e.g. by removal of a peptide from the N and/or C-terminal ends. Such fragments may be derived from the sequence of Sequence



(1) (optionally together with Sequence (2)) or may be derived from a functionally equivalent peptide as described above. Preferably such fragments are between 6 and 30 residues in length, e.g. 6 to 25 or 10 to 15 residues.

5  
10  
Preferably functional variants according to the invention have an amino acid sequence which has more than 70%, e.g. 75 or 80%, preferably more than 85%, e.g. more than 90 or 95% homology to Sequence (1), or Sequence (1) when attached to Sequence (2), (according to the test described hereinafter).

15  
20  
25  
In connection with amino acid sequences, "sequence identity" refers to sequences which have the stated value when assessed using ClustalW (Thompson et al., 1994, supra) with the following parameters:  
Pairwise alignment parameters - Method: accurate,  
Matrix: PAM, Gap open penalty: 10.00, Gap extension penalty: 0.10;  
Multiple alignment parameters - Matrix: PAM, Gap open penalty: 10.00, % identity for delay: 30, Penalize end gaps: on, Gap separation distance: 0, Negative matrix: no, Gap extension penalty: 0.20, Residue-specific gap penalties: on, Hydrophilic gap penalties: on,  
Hydrophilic residues: GPSNDQEKR. Sequence identity at a particular residue is intended to include identical residues which have simply been derivatized.

Preferably peptides of the invention have the general formula I:

30             $Y_1-X_1-X_2-X_3-W-M-X_4-X_5-X_6-X_7-Y_2$             (I)

wherein

35            the sequence  $X_1$  to  $X_7$  is an amino acid sequence comprising at least 9 amino acids, which may optionally be interrupted by one or more (preferably one or two) amino acid residues between one or more of the 9 amino acid positions defined

herein;

Y<sub>1</sub>, which may be present or absent, is a moiety attached to X<sub>1</sub> (or X<sub>2</sub> when X<sub>1</sub> is absent) preferably via the available amino group on X<sub>1</sub> (or X<sub>2</sub>), but  
5 alternatively via the side-chain of X<sub>1</sub> (or X<sub>2</sub>), wherein Y<sub>1</sub> is preferably a peptide of 50 amino acids or less which is optionally substituted;

Y<sub>2</sub>, which may be present or absent, is a moiety attached to X<sub>7</sub>, preferably via the carboxyl group on  
10 X<sub>7</sub>, but alternatively via the side-chain of X<sub>7</sub>, wherein Y<sub>2</sub> is preferably a peptide of 50 amino acids or less which is optionally substituted;

X<sub>1</sub>, which may be present or absent, is one or more amino acids, and is preferably W, T, PE, KQI, VV,

15 PQT, H or RI;

X<sub>2</sub> is an amino acid with an aromatic side chain, preferably Y, F or W;

X<sub>3</sub> is the amino acid P or D;

X<sub>4</sub> is an amino acid with a basic side chain,  
20 preferably K, R or H;

X<sub>5</sub> is an amino acid with a charged side chain, preferably a basic side chain, especially preferably K, R, E, H, D, N or Q;

X<sub>6</sub> is an amino acid with a charged side chain,  
25 preferably a basic side chain, especially preferably K, R, E, H, D, N or Q;

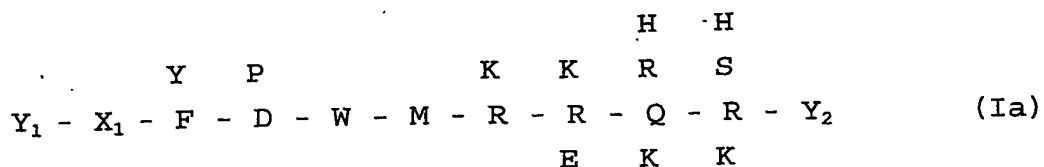
X<sub>7</sub> is an amino acid with a basic side chain or serine, especially preferably H, S, R or K;

or a functionally equivalent derivative, variant or  
30 fragment thereof.

As mentioned above, Y<sub>1</sub> and/or Y<sub>2</sub> may be substituted by a further moiety. Such moieties may be added to aid the function of the peptide, its targeting or its synthesis, capture or identification, e.g. a label (e.g.  
35 biotin) or lipid molecules.

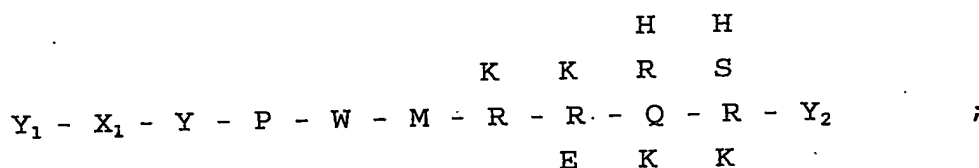
In the above sequence, X<sub>1</sub>-X<sub>4</sub> forms the hexapeptide sequence.

Preferred peptides of formula I, have the formula  
Ia:

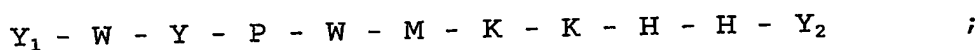


wherein  $\text{Y}_1$ ,  $\text{X}_1$  and  $\text{Y}_2$  are as defined hereinbefore.

Especially preferably formula Ia has the sequence:



especially preferably,



wherein  $\text{Y}_1$ ,  $\text{X}_1$  and  $\text{Y}_2$  are as defined hereinbefore.

Preferably  $\text{Y}_2$  is a cell penetration moiety, wherein said moiety is preferably a peptide (which may optionally be substituted, e.g. with a label or attachment moiety). A cell penetration moiety may alternatively be associated with a peptide of general formula I, e.g. may encapsulate or form a complex with said peptide, e.g. by using liposomes for lipofection or polycations or cationic lipids. "Associated with" as used herein refers to the moiety being attached to, or connected in some way, to the peptide.

As used herein a "cell penetration moiety" refers to a molecule or collection of molecules which assist or facilitate entry of the molecule to which it is attached

into a cell. A variety of such moieties are well-known in the art and include peptides such as penetratins, tat-derived proteins, signal-sequence based peptides as well as synthetic and/or chimeric cell-penetrating peptides such as transportan or model amphipathic peptides (Lindgren et al., 2000, TIPS, 21, p99-103 and Derossi et al., 1998, Trends C. Biol., 8, p84-87). Preferably said cell penetration moiety acts by a receptor-independent mechanism.

In a preferred feature said cell penetration moiety is a peptide based on the penetratin sequence and has the following general formula II:



wherein  $X_8$  is an amino acid and is R or Q; or a functionally equivalent derivative, variant or fragment thereof. Preferably a variant of the formula II sequence is a peptide in which one or more of said K residues in the formula II sequence is replaced with an R residue, one or more of said R residues in the formula II sequence is replaced with a K residue and/or one or more of said I residues in the formula II sequence is replaced with an L residue. Especially preferably, said variant has the form:

QIRIWFQNRMRMKWKK;  
QIKIWFQNKRMKWKK;  
QIKIWFQNKMKWKK;  
QIRIWFQNRKMKWKK;  
QIRIWFQNRMRWKK;  
QIRIWFQNRMRKWRK;  
QIRIWFQNRMRKWKR;  
QIRIWFQNRMRKWRR;  
QIRIWFQNRMRKWKK;  
QIKIWFQNRMRKWRK;  
QIRIWFQNKRMKWRK;

QIKLWFQNRRMKWKK;  
QLKLWFQNRRMKWKK; or  
QLRIWFQNRRMKWKK.

5            Preferably the peptide of formula II is the Y<sub>2</sub> group of the peptides of formula I or Ia.

          "Peptides" as referred to herein are molecules with less than 100 amino acid residues, but are preferably shorter, e.g. less than 50, e.g. less than 30 residues  
10    in length, preferably from 8 to 25 residues in length.

          Peptides as described above for use in accordance with the invention may be prepared by conventional modes of synthesis including genetic or chemical means. Chemical synthesis may be performed by methods well  
15    known in the art involving cyclic sets of reactions of selective deprotection of the functional groups of a terminal amino acid and coupling of selectively protected amino acid residues, followed finally by complete deprotection of all functional groups.  
20    Synthesis may be performed in solution or on a solid support using suitable solid phases known in the art.

          The present invention also extends to antibodies (monoclonal or polyclonal) and their antigen-binding fragments (eg. F(ab)<sub>2</sub>, Fab and Fv fragments ie. fragments  
25    of the "variable" region of the antibody, which comprises the antigen binding site) directed to peptides as defined hereinbefore, ie. which bind to epitopes present on the peptides and thus bind selectively and specifically to such peptides, and which may be used in  
30    the methods of the invention.

          Peptides described above may be used to block interactions of PBX with its binding partners, e.g. HOX, and preferably thereby prevent the binding of HOX to its target DNA. Thus in a further aspect the present  
35    invention provides use of a peptide as described hereinbefore to reduce or inhibit binding of PBX to a binding partner, preferably HOX or use of such peptides

to reduce or inhibit binding of HOX to its target DNA.

As referred to herein "binding" refers to the interaction or association of at least two moieties in a reversible or irreversible reaction, wherein said  
5 binding is preferably specific and selective,

As used herein a "binding partner" refers to a molecule which recognizes and binds specifically (ie. in preference to binding to other molecules) to its binding partner. Such binding pairs when bound together form a  
10 complex.

A "reduction in binding" refers to a decrease in binding, e.g. as manifest by an increased concentration of one of the binding pair required to achieve binding. Reduction includes a slight decrease as well as absolute  
15 abrogation of specific binding. A total reduction of specific binding is considered to equate to a prevention of binding. "Inhibition" refers to competitive interference of the binding of the binding partners by the peptide, which serves to reduce the partners'  
20 binding.

Agents which prevent or reduce PBX-dependent transcription regulation, have surprisingly been found to have advantageous effects on aberrant cell division as described hereinbefore. Such agents are preferably  
25 those which prevent, reduce or inhibit the binding of PBX to its binding partners, especially preferably the binding between PBX and HOX (such as antagonists of the interaction between HOX and PBX, e.g. the peptides described hereinbefore). However, suitable agents also  
30 include those that affect binding of the transcription factors to the target DNA, e.g. which block the interaction of PBX or its binding partner, such as HOX, to the target DNA. Especially preferably such agents prevent HOX-dependent transcription regulation.

35 Whilst not wishing to be bound by theory, it is believed that antagonists of HOX:PBX binding prevent the interaction between multiple important HOX:PBX protein

binding partners, and the HOX proteins are therefore unable to act as transcription factors on the genes to which they bind. The failure to regulate expression of these genes has the effect of reducing or preventing the excessive cell division. Similarly any moiety which prevents or reduces PBX-dependent transcription regulation, e.g. blocks the interaction of HOX with its target DNA, may be expected to have similar effects.

In a further aspect therefore, the present invention provides a method of reducing aberrant cell division wherein said cells are administered an agent which prevents or reduces PBX-dependent transcription regulation (preferably which reduces or prevents binding of PBX to a binding partner, preferably to HOX (preferably HOXB4, HOXB8 or HOXA9) or reduces or prevents binding of HOX to its target DNA, preferably an antagonist, especially preferably an antagonist of the interaction between HOX and PBX, especially preferably a peptide as described hereinbefore (and which preferably inhibits HOX-dependent transcription regulation).

Agents which are suitable for this purpose include antagonists of the interaction between HOX and/or PBX and the DNA target to which they bind, antagonists of the interaction between PBX and its binding partners, especially HOX proteins, or agents which impair the binding ability of HOX/PBX or the target DNA, e.g. which block relevant sites or cause structural changes at relevant sites on HOX/PBX or the target DNA or reduce the number of molecules available for binding (which may be achieved by for example modifying the expression/expressed product of PBX/HOX). Preferably however, antagonists are employed.

As described herein, "aberrant cell division" refers to cell division above the normal level (ie. abnormal cell division) considered appropriate under the conditions which exist. Markers of aberrant cell division. More particularly, such aberrant cell

division may be present in certain conditions or diseases/disorders as described hereinafter, such as cancer.

"Reducing" cell division refers to reducing the rate of cell growth. Preferably cell growth is reduced to less than 0.5, especially preferably less than 0.25, e.g. less than 0.1 relative to control growth (without the agent) over the same time period (wherein control growth = 1). In a preferred aspect reduced cell division encompasses cell death/lack of viability which may occur in addition, or as an alternative to the reduction in cell growth. When cell death occurs preferably more than 50% of the existing cells, preferably more than 75% of the cells are destroyed.

As described herein "PBX-dependent transcription regulation" refers to activation or suppression of the transcription of genes by processes in which PBX plays a pivotal role, e.g. acts as a cofactor in the transcription regulatory complexes. Prevention or reduction refers to a measurable change in the extent of transcription. Prevention equates to a reduction in transcription to undetectable levels.

"PBX" refers to the family of pre-B-cell transformation related genes and includes genes encoding extradenticle homeoprotein proteins and homologues of the Drosophila extradenticle gene, such as genes in vertebrates. Vertebrate PBX proteins are transcription factors that contain a homeodomain (Mann et al., 1996, supra).

"HOX" refers to homeobox genes which contain a sequence which encodes a homeodomain of about 60 amino acids and a sequence which encodes the hexapeptide sequence N-terminal to the homeodomain (Morgan et al., 2000, TIG, 16(2), p66-67 and Krumlauf, 1994, Cell, 78(2), p191-201). The HOX proteins are transcription factors that act to define anterior-posterior development in early development. Such PBX or HOX genes



or proteins as described herein include homologues present in any multicellular animal, but are preferably from vertebrates, e.g. from mammals, especially preferably from humans.

5       "Target DNA" refers to the gene containing the regulatory region to which PBX, HOX or any member of the transcription regulation complex containing such proteins, binds.

10       As referred to herein, an "antagonist" is a molecule or complex of molecules which by virtue of structural similarity to one molecule of a binding pair competes with that molecule for binding to the other molecule of the binding pair. Antagonists for use in the invention include antagonists of the interaction  
15       between HOX and PBX which prevent or reduce binding between those entities. Preferred antagonists are peptides, antibodies, or anti-idiotypes in which these molecules bind to, or compete with the binding site on HOX or PBX. Preferably the antagonists compete by  
20       mimicking the PBX binding site on HOX, ie. binding to PBX, e.g. peptides as described hereinbefore.

      Other antagonists include those which prevent or reduce binding between HOX and its target DNA. HOX proteins are known to bind to a 6 base pair consensus  
25       sequence NNATTA on their target DNA and antagonists of this binding, e.g. oligonucleotides which are complementary to that sequence (e.g. sets of oligonucleotides with variability at 2 bases to accommodate the variability in the consensus sequence  
30       described above) are suitable as agents for the above described purpose

      Such methods may be performed *in vitro*, *in vivo* or *ex vivo*. Conveniently such methods are performed *in vivo* by the administration of said agent, preferably an  
35       antagonist as described hereinbefore, to a human or non-human animal to treat or prevent a condition or disorder in which aberrant cell division occurs, e.g. cancer or a

non-cancerous growth such as myelodysplastic syndrome (MDS). Alternatively expressed, the present invention provides the use of an agent as described hereinbefore, preferably an antagonist, especially preferably an antagonist of the interaction between HOX and PBX, e.g. a peptide as described hereinbefore, in the manufacture of a medicament for the treatment or prevention of a condition or disorder in which aberrant cell division occurs.

As referred to herein a "disorder" or a "disease" refers to an underlying pathological disturbance in a symptomatic or asymptomatic organism relative to a normal organism, which may result, for example, from infection or an acquired or congenital genetic imperfection.

A "condition" refers to a state of the mind or body of an organism which has not occurred through disease, e.g. the presence of a moiety in the body such as a toxin, drug or pollutant.

As defined herein "treatment" refers to reducing, alleviating or eliminating one or more symptoms of the condition or disorder which is being treated, relative to the symptoms prior to treatment. For example, symptoms which may be affected include tumour size or numbers of cancerous cells in a given sample (or reduced stem cell numbers as described hereinafter).

"Prevention" of a condition or disorder refers to delaying or preventing the onset of a condition or disorder or reducing its severity, as assessed by the appearance or extent of one or more symptoms of said condition or disorder.

As an alternative to performing the methods *in vivo*, such methods may be performed *in vitro*, e.g. to reduce the cell division of, or eliminate, cells undergoing aberrant cell growth, in a sample. Appropriate culture conditions are as described for other methods of the invention as described hereinafter.

This is particularly useful in cell samples containing both normal and aberrant cells in which aberrant cells may be controlled/removed and the sample containing the normal cells used for subsequent procedures, e.g. returned to the donor body. This may be useful to, for example, eliminate aberrant haematopoietic blood cells from a blood sample of a patient, e.g. leukaemic cells, and the remaining cells may then be returned to the body of that patient.

Thus in a yet further aspect the present invention provides a method of reducing aberrant cell division (preferably of reducing the growth, preferably involving the death and hence reducing the number, of cancer cells) in cells in a sample, wherein an agent as described hereinbefore is administered to said sample. In a method for treating patients suffering from a disorder or condition typified by aberrant cell division (or preventing the same), said sample may be harvested from said patient and then returned to that patient as described hereinafter.

In this context, a "sample" refers to any material obtained from a human or non-human animal, including embryonic, foetal, immature and adult stages of said animal, which contains cells undergoing aberrant cell division and include tissues and body fluids. "Body fluids" in this case include in particular blood, spinal fluid and lymph and "tissues" include tissue obtained by surgery or other means.

Preferably the aberrant cell division occurs in cells from eukaryotic organisms which may be any eukaryotic organisms such as human beings, other mammals and animals, birds, insects and fish.

Preferred non-human animals from which cells may be derived or on which methods of the invention may be conducted include, but are not limited to mammals, particularly primates, domestic animals, livestock and laboratory animals. Thus preferred animals include

mice, rats, chickens, frogs, guinea pigs, cats, dogs, pigs, cows, goats, sheep, horses. Particularly preferably the cells are derived from, and the methods used to treat, or be prophylactic in, humans.

5            Preferably the cells undergoing aberrant cell division are cancer cells and the disorder to be treated or prevented is a cancer. Especially preferably said cancers are malignant or pre-malignant or benign tumours and include carcinomas, sarcomas, gliomas, melanomas and  
10           lymphomas, including cancers of the bladder, kidney, pancreas, brain, head and neck, breast, gut, prostate, lung and ovary and leukaemias and lymphomas, especially preferably leukaemias.

            Thus in a preferred aspect, the present invention  
15           provides a method of treating or preventing cancer, e.g. leukaemia in a human or non-human animal wherein said animal is administered an agent, preferably an antagonist, as described hereinbefore.

            Agents which prevent or reduce PBX-dependent  
20           transcription regulation have also been found to have beneficial effects on stem cells.

            As described in the Examples herein it has now been found that prevention of PBX-mediated transcription regulation results in reduced, but continued, cell  
25           division and the appearance of molecular markers of differentiation (e.g. CD38). However on removal of the agent blocking that transcriptional regulation cells reverted to stem cells as assessed by the appearance of molecular markers (e.g. HOXB4, HOXB8, HOXA9, AC133),  
30           thus reflecting pluripotency of the cells. Whilst not wishing to be bound by theory, it is believed that despite the appearance of markers of differentiation/maturation, no phenotypic changes symptomatic of differentiation occur and the cells  
35           instead have a significantly reduced rate of cell cycling while the agent is being administered. On removal of the agent, the cells revert to stem cells.

This has a number of applications which include maintenance or expansion of stem cells (e.g. in culture), for example for temporary storage of said cells, with possible expansion during that storage period. Such cells may then, for example, be used in clinical applications in which the addition of stem cells is desirable, e.g. to patients that have reduced numbers of stem cells and/or the ability to produce certain differentiated cell types; due to, for example, age, disease (e.g. cancers or autoimmune disease), congenital factors, environmental influences or contaminants and/or administered chemicals. In particular stem cells may be harvested from a patient prior to chemotherapy or radiotherapy and maintained and/or expanded and returned to that patient after chemotherapy or radiotherapy. As an alternative example the stem cells may be used to provide cells from which a particular differentiated cell may be formed, e.g. neuronal cells, particularly in adult recipients where such suitable stem cells are absent or only low levels are present. The recipient of the stem cells is preferably also the donor, but may also be a different individual.

Cells may also be maintained *ex vivo* or *in vivo*, for example to maintain viability during treatment that might normally affect their viability, e.g. during chemo- or radio-therapy. Agents as described herein, e.g. peptides of the invention, slow the cell cycle of stem cells and thus reduces their susceptibility to damage by such treatments.

Thus in a further aspect, the present invention provides a method of maintaining or expanding stem cells, wherein said method comprises at least the step of contacting said cells with an agent as described hereinbefore, preferably an antagonist, especially preferably an antagonist of the interaction between HOX and PBX, e.g. a peptide as described hereinbefore. This

method may be used to maintain pluri- or toti-potency of the stem cells.

Preferably this method is performed *in vitro* or *ex vivo*, in culture, in which case the method may contain  
5 an initial step of harvesting stem cells from a donor. However, the method may also be used *in vivo* to maintain or improve the numbers of stem cells in an individual, particularly during exposure to agents or treatments that might cause stem cell damage. In such  
10 circumstances, the present invention provides a method of maintaining or expanding stems cells in a patient wherein said patient is administered an agent as described hereinbefore, preferably an antagonist, especially preferably an antagonist of the interaction  
15 between HOX and PBX, e.g. a peptide as described hereinbefore.

"Maintaining" the cells refers to maintaining the viability of a large proportion of the starting, e.g. harvested, cells with minimal cell division, during the  
20 course of the treatment or culture period.

"Expanding" the cells refers to at least some cell division, preferably significant cell division, to increase the numbers of cells during the course of treatment, or culture.

25 "Stem cells" as referred to herein are undifferentiated cells which are capable of differentiating into various cells, e.g. various blood cell types and include haematopoietic (e.g. found in the bone marrow) and neural and hepatic stem cells,  
30 embryonic stem cells and embryonic germ cells and encompass both pluri- and toti-potent cells. Embryonic cells are considered to be those cells derived from the inner cell mass of the blastocyst and embryonic germ cells are those cells isolated from the primordial germ  
35 cell of the gonadal ridge of the 5 to 10 week old foetus. Preferably said cells are derived from eukaryotic organisms as described previously.

As referred to herein "culture" refers to the growth or maintenance of the cells in a controlled artificial environment, ie. *ex vivo*. Standard techniques for culture of cells are well known.

5 Preferably cells are cultured at 37°C, 5% CO<sub>2</sub> in a humidified atmosphere in a standard culture medium. Preferably said culture is conducted for at least 2 hours, preferably more than 24 hours, e.g. between 24 hours and 8 weeks.

10 "Contacting" as used herein refers to any suitable technique which allows the agent to have access, and thus the possibility of binding, to cells in the sample, e.g. by application to the culture medium.

15 After the cells have been maintained or expanded, the agent may be removed to recover pluri- or toti-potency. When the method is performed *in vivo* this may be achieved by ceasing administration and allowing the body to clear the agent. *In vitro* or *ex vivo*, the agent is removed from the culture medium, e.g. by washing and  
20 replacement with fresh medium.

Thus alternatively described the invention provides a method of maintaining or expanding stem cells and or obtaining pluri- or toti-potent stem cells, in culture, preferably an expanded population of said cells, wherein  
25 said method comprises at least the steps of:

a) contacting said cells in culture with an agent which reduces or prevents PBX-dependent transcription regulation as described hereinbefore, preferably an antagonist, especially preferably an antagonist of the  
30 interaction between HOX and PBX, e.g. a peptide as described hereinbefore;

b) culturing said cells in the absence of said agent. It should be noted that the peptide becomes degraded within a few days during culture and thus active peptide  
35 is depleted. Thus, step b) may be performed without any prior washing if sufficient time has lapsed for degradation to occur. As mentioned previously,

appropriate culture times are at least 2 hours, preferably more than 24 hours, e.g. between 24 hours and 8 weeks.

The method may contain an initial step of harvesting stem cells from a donor. Cells obtained by this and other methods of the invention comprise further aspects of the invention as does their use as a medicament.

The cells thus prepared by the above described *in vitro* or *ex vivo* methods may then be administered to an individual in need of such stem cells. Optionally the cells may be modified prior to transplant, e.g. during the course of culturing or just prior to transplanting, e.g. by genetic modification, e.g. for gene transfer or to import a function not previously present in said cells, e.g. to compensate for a genetic deficit, e.g. by providing a missing factor, e.g. adenosine deaminase (ADA).

Thus in a yet further aspect, the present invention provides a method of treating an individual in need of stem cells wherein stem cells prepared according to the above described method are administered to said individual.

Preferably said individual in need of said stem cells is an individual who has (or will have) lower than normal or desirable levels of such cells, which condition may exist normally, e.g. through age or as a result of external factors e.g. through chemotherapy or radiotherapy. Especially preferably, said stem cells are derived from the recipient individual.

Thus in a preferred feature the present invention provides a method of improving the number of stem cells in a recipient individual wherein said method comprises at least the steps of:

- a) harvesting stem cells from a donor,
- b) culturing said stem cells according to the methods described hereinbefore;



c) administering said cultured stem cells to said recipient individual.

Preferably said method is a method of improving the number of stem cells in an patient subject to

5 chemotherapy or radiotherapy, wherein said method comprises at least the steps of:

a) harvesting stem cells from said patient prior to chemotherapy or radiotherapy,

b) culturing said stem cells according to the methods

10 described hereinbefore;

c) administering said cultured stem cells to said patient after completion of chemotherapy or radiotherapy.

Alternatively described, harvesting step a) in the

15 methods above may be absent and step b) may comprise culturing stem cells harvested from the donor according to the methods described hereinbefore. Said cells may be harvested by obtaining a sample of cells, tissue or body fluid from said donor and optionally extracting the

20 cells therefore.

As used herein a "sample" refers to any material obtained from the donor, e.g. human or non-human animal, including embryonic, foetal, immature and adult stages of said animal, which contains stem cells and includes,

25 tissues and body fluids. "Body fluids" include blood and spinal fluid. "Tissue samples" include tissue obtained by surgical interventions (e.g. bone marrow or liver) or by other means e.g. placenta and umbilical cord. The animals from which cells are derived or to

30 which the methods are applied are preferably as described hereinbefore in connection with the methods of reducing aberrant cell division.

As used herein reference to "improving the number of stem cells" refers to increasing the number of stem

35 cells to be added (preferably of the particular type to be added, e.g. haematopoietic stem cells) relative to the number present in the individual at the time at

which administration would occur. Thus in the case of a patient subject to chemotherapy or radiotherapy the observed improvement is in the number of stem cells in a patient post-chemotherapy or post-radiotherapy. An improvement may also consist of the addition of certain stem cells previously absent or present in very low numbers, e.g. neuronal stem cells.

Alternatively expressed, the present invention provides the use of an agent (preferably an antagonist as described hereinbefore) in the preparation of a medicament for the treatment or prevention of conditions or disorders typified by a need for stem cells, preferably in treating or preventing conditions or disorders in which stem cell numbers are lower than normal, e.g. due to chemotherapy or radiotherapy, or in conditions in which the provision of stem cells may allow the production of one or more particular differentiated cells that are absent or present in abnormally low numbers, or lower numbers than desired, at the site of interest.

Conditions or disorders in which stem cell numbers are lower than normal include autoimmune disorders, radiotherapy, chemotherapy and certain viral infections. Conditions in which the use of stem cells by transplantation may provide appropriate differentiated cells which are absent or present at lower than normal or lower than desired levels include Alzheimer's disease, Parkinson's disease and other age-related disorders or conditions (including cosmetic treatments), multiple sclerosis, spinal cord injury, diabetes, chronic heart disease, end-stage kidney disease, liver failure and in which stem cells are used to replace destroyed or dysfunctional cells. Prevention of such conditions or disorders may be achieved by maintaining stem cells in a protected state by the use of agents as described hereinbefore.

The present invention further provides the use of

cells prepared by the methods described hereinbefore in the preparation of a medicament for the treatment of conditions or disorders typified by a need for stem cells, as described above.

5           It should be noted that due to the effects of the  
aforementioned agents on aberrant cell division, even  
samples of stem cells containing such aberrant cells may  
be used and a dual effect of reducing the aberrant  
division while expanding the stem cells may be achieved.  
10       Thus the aforementioned agents may be used *in vitro*, *ex*  
*vivo* or *in vivo* to protect normal stem/progenitor cells  
whilst eliminating cells undergoing aberrant cell  
growth. This is particularly applicable to  
haematopoietic cells, e.g when treating  
15       leukaemia/lymphoma.

          Thus in a further preferred aspect the present  
invention provides a method of treating or preventing a  
condition or disorder in which aberrant cell division  
occurs, e.g. cancer, in a human or non-human animal,  
20       wherein said method comprises administering an agent,  
preferably an antagonist as described hereinbefore,  
wherein said agent is capable of both reducing said  
aberrant cell division and maintaining or expanding stem  
cells of said animal.

25       As described above, agents which reduce or prevent  
PBX-dependent transcription regulation, particularly  
HOX:PBX antagonists and particularly peptides as  
described hereinbefore have various clinical  
applications and thus a further aspect of the invention  
30       provides pharmaceutical compositions containing such  
agents. The use of these agents as a medicament forms a  
further aspect of the invention.

          Thus, in a further aspect the present invention  
provides a pharmaceutical composition comprising an  
35       agent which reduces or prevents PBX-dependent  
transcription regulation as described hereinbefore,  
preferably an antagonist, especially preferably an

antagonist of the interaction between HOX and PBX, e.g. a peptide as described hereinbefore, and a pharmaceutically acceptable excipient, diluent or carrier.

5           Pharmaceutical compositions as described herein for use as a medicament, preferably for use in treating or preventing disorders or conditions typified by aberrant cell division, or disorders or conditions typified by a need for stem cells, such as the conditions described  
10       hereinbefore, and methods of treatment or prophylaxis using such compositions and use of said agents for the preparation of a medicament for treating or preventing such disorders or conditions, form further aspects of the invention.

15           "Pharmaceutically acceptable" as referred to herein refers to ingredients that are compatible with other ingredients of the compositions as well as physiologically acceptable to the recipient.

          Pharmaceutical compositions according to the  
20       invention may be formulated in conventional manner using readily available ingredients. Thus, the active ingredient (e.g. the peptide), may be incorporated, optionally together with other active substances, with one or more conventional carriers, diluents and/or  
25       excipients, to produce conventional galenic preparations such as tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments, soft and hard gelatin capsules,  
30       suppositories, sterile injectable solutions, sterile packaged powders, and the like.

          Compositions may additionally comprise molecules which assist or augment the action of the agents, preferably the peptides, described hereinbefore, e.g.  
35       cytotoxic agents such as antimetabolites, alkylating agents, cytotoxic antibiotics, topoisomerase I and/or II inhibitors, vinca alkaloids and monoclonal antibodies.

Alternatively viewed, the agents as described herein may be used to assist or augment the action of agents used for conventional treatments, e.g. cytotoxic agents, to reduce their side effects, e.g. by protection of stem cells during treatment.

If required, the compositions may also contain targeting moieties attached to the active ingredient, e.g. a ligand which binds specifically and selectively to an endogenous receptor to allow targeting to a particular cell type or location, such as targeting to lymphocytes, monocytes, macrophages, endothelial cells, epithelial cells, blood cells, erythrocytes, platelets, eosinophils, neutrophils, natural killer cells, dendritic cells, brain cells, heart cells, lung cells, islet cells, kidney cells, cancer cells, hormonal gland cells, skin, bone, joints, bone marrow, gastric mucosa, lymph nodes, Peyers patches, the omentum and other appropriate tissues.

Examples of suitable carriers, excipients, and diluents are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, aglinates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, water, water/ethanol, water/glycol, water/polyethylene glycol, propylene glycol, methyl cellulose, methylhydroxybenzoates, propyl hydroxybenzoates, talc, magnesium stearate, mineral oil or fatty substances such as hard fat or suitable mixtures thereof. The compositions may additionally include lubricating agents, wetting agents, emulsifying agents, suspending agents, preserving agents, sweetening agents, flavouring agents, and the like. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

Compositions may be in an appropriate dosage form,

for example as an emulsion or in liposomes, niosomes, microspheres, nanoparticles or the like.

Administration of agents or compositions of the invention may take place by any of the conventional routes, e.g. orally, rectally or parenterally, such as by intramuscular, subcutaneous, intraperitoneal or intravenous injection, infusion, inhalation or topical administration, both to internal or external body surfaces etc. depending on the condition or disorder to be treated or prevented, optionally at intervals, e.g. 3 or more applications at 3-5 day intervals. Conveniently intravenous injection is used.

The active ingredient in composition of the invention may comprise from about 0.01% to about 99% by weight of the formulation, preferably from about 0.1 to about 50%, for example 10%. The compositions are preferably formulated in a unit dosage form, e.g. with each dosage containing from about 0.01mg to about 1g of the active ingredient, e.g. 0.05mg to 0.5g, for a human, e.g. 1-100mg.

The precise dosage of the active compound to be administered and the length of the course of treatment will, of course, depend on a number of factors including for example, the age and weight of the patient, the agent to be used, the purpose of the treatment, the specific condition requiring treatment or prevention and its severity, and the route of administration. Generally however, an effective dose may lie in the range of from about 1 $\mu$ g/kg to about 10mg/kg, e.g. from about 1mg to 0.2g of the agent per day, depending on the animal to be treated and the dosage form, taken as a single dose. Thus for example, an appropriate daily dose for an adult may be from 0.5mg to 0.5g per day, e.g. 1 to 100mg of the polypeptide per day. In smaller animals the concentration range may be different and can be adjusted accordingly.

For *in vitro* or *ex vivo* use a concentration range

of 1µg/ml to 10mg/ml for the agent, e.g. the peptide as described hereinbefore, is suitable.

The following examples are given by way of illustration only in which the Figures referred to are as follows:

Figure 1 shows the effect of HXP peptide on cell growth of immature blood stem cells (AC133<sup>+</sup> cells) and on leukaemic cell lines KG1a, KG1, HL60 and U937 as a function of the time after HXP administration;

Figure 2 shows the effect of HXP peptide on the cell cycle of cells (AC133<sup>+</sup>, KG1a, KG1, HL60 and U937) to which it is administered as a function of the time after HXP administration;

Figure 3 is similar to Figure 1 and shows the longer term effects of HXP peptide on the cell growth of AC133<sup>+</sup>, KG1a, KG1 and U937 cells;

Figure 4 shows the results of RT-PCR to detect various markers of stem/progenitor cells or markers of maturation/differentiation in KG1a cells after 7 days of treatment with HXP peptide;

Figure 5 shows the effect of HXP peptide on the cell growth of AC133<sup>+</sup> cells in which -▲- is the control with no HXP added, -■- shows cells to which HXP was added at days 0, 7 and 14, -♦- shows cells to which HXP peptide was added at day 0, but withdrawn at days 7 and 14, and -X- shows cells to which HXP peptide was added at day 0 and on day 7 HXP peptide was withdrawn and HOX B4 protein added;

Figure 6 shows the results of RT-PCR to detect various markers of stem/progenitor cells or markers of maturation/differentiation in the cells examined as

described in Figure 5. The corresponding graph from Figure 5 is shown in each case. a) control growth curve; b) addition of HXP at day 0, 7 and 14; c) addition of HXP peptide at day 0 and withdrawal of HXP at day 7 and 14; and d) initial addition of HXP peptide at day 0, simultaneous withdrawal of HXP peptide and addition of the HOXB4 protein at day 7; and

Figure 7 shows the results of cross-linking PBX proteins from KG1a cells in which in the Western blot a) protein is probed with anti-PBX antibodies and b) with anti-beta actin antibodies, and no cross-link or cross-link refers to the performance or absence of cross-linking after cell lysis and lane 1 contains untreated cells, lane 2 contains cells treated with control peptide and lane 3 contains cells treated with HXP4.



Example 1: Effect of HXP peptide on growth, cell cycle and viability in vitro

In this example HXP peptide, a peptide generated to mimic the conserved region on HOX proteins, specifically the hexapeptide region of HOXB-4, was used in *in vitro* assays to determine its effects on cell growth, cell cycle and cell death on a variety of normal and abnormal cell lines or primary cell cultures.

Methods

1. *HXP Peptide*

The HXP peptide has the following sequence:  
(N-terminal) WYPWMKKHHRQIKIWFQNRMRMKWK (C-terminal)  
and was prepared by routine chemical synthesis.

2. *Blood Stem Cells culture*

2.1. *Umbilical Cord Blood collection and Mononuclear cell isolation*

Umbilical Cord Blood (UCB) specimens were collected from full-term deliveries scheduled for elective caesarian sections following hospital ethical regulations.

Samples were diluted 1 in 4 in PBS supplemented with a citrate-based anti-coagulant (0.6% ACD-A, Baxter, France) and Bovine Serum Albumin (0.5% fraction V, Sigma Aldrich, UK) at pH=7.4 and referenced as "ACD-A buffer".

Diluted UCB was carefully overlaid in a 1:4 ratio onto a research grade Ficoll-Paque solution ( $d:1.077\text{g/cm}^3$ , Pharmacia Biotech, Sweden) prior to Centrifugation (400g, 30 minutes,  $22^\circ\text{C}$ ). The mononuclear cells (MNC) layer was extracted, washed twice in ACD-A Buffer, pelleted (400g, 10 minutes) before resuspending in ACD-A buffer and cell aliquots taken for cell viability / enumeration using trypan blue (Sigma Aldrich).

## 2.2. AC133<sup>+</sup> cell immunomagnetic selection

AC133<sup>+</sup> cells were obtained from MNC after immunomagnetic separation using the AC133 mini-MACS selection kit (Miltenyi Biotec, Germany): labelling volume 500 $\mu$ l/10<sup>8</sup> cells in ACD-A buffer containing Fc Receptor-blocking reagent (100 $\mu$ l, 5min incubation, 4°C) before adding colloidal super-paramagnetic MACS MicroBeads conjugated to monoclonal mouse anti-human AC133/1 antibody (100 $\mu$ l IgG1 isotype, 25min incubation, 4°C). Cells were then washed (5ml ACD-A buffer, 400g, 10min, 4°C) before resuspended cells in 500 $\mu$ l ACD-A buffer were applied to a chilled MACS positive selection column (MS+/RS+) on a magnet. The column was rinsed with cool ACD-A buffer (4x500 $\mu$ l) and the AC133<sup>+</sup> cell population retained at 4°C. After magnet removal AC133<sup>+</sup> cells were eluted with 1ml of cold ACD-A buffer. The AC133<sup>+</sup> cell fraction was reapplied to a new column, prior to cell enumeration and viability assays.

## 2.3. Short-term ex-vivo AC133<sup>+</sup> cell expansion cultures with HXP peptide

AC133<sup>+</sup> cells were seeded in duplicate in liquid culture system at 2-4x10<sup>4</sup> cells/ml. The liquid culture system consisted (in 1.5ml total) of Iscove Modified Dulbecco's Medium (Life Technologies, UK) supplemented with Foetal Calf Serum (10%, Sigma Aldrich) and gentamycin (50 $\mu$ g/ml Life Technologies). Culture systems were supplemented with growth factors 'TPOFLK' (Thrombopoietin 10ng/ml, Flt-3 Ligand 50ng/ml) and HXP peptide (20 $\mu$ g/ml). AC133<sup>+</sup> cells were also cultured with TPOFLK and the control peptide (WAPWEDDHHRQIKIWFQNRMKWKK, same concentration as HXP). Every 7 days, the medium was changed and when required: (i) HXP peptide was added (concentration maintained) or (ii) withdrawn, (iii) Hox B4 protein (20 $\mu$ g/ml, recombinant full length *Xenopus laevis* sequence) was added. Cells were cultured for up to 18 days at 37°C, 5% CO<sub>2</sub> in humidified atmosphere. Cultured

cells were then counted at various time points using trypan blue exclusion method described above.

### 3. *Leukaemic cell lines culture*

5

KG1a, KG1, HL60 and U937 cell lines were obtained from the ATCC (catalogue Nos. CCL-246.1, CCL-246, CCL-240 and CRL-1593.2, respectively. Cell lines were seeded at  $1-5 \times 10^5$  cells/ml, in liquid culture system made of RPMI-1640 medium (Life Technologies, UK) supplemented with Foetal Calf Serum (10% Sigma Aldrich) and gentamycin (50 µg/ml Life Technologies) and at least in duplicate. When required the HXP peptide was added to the medium (20 µg/ml). Cell lines were cultured for up to 7 days at 37°C, 5% CO<sub>2</sub> in humidified atmosphere. Cultured cells were then counted at various time points using trypan blue exclusion method described above.

20

### 4. *Cell cycle analysis*

25

30

Cells to be investigated were harvested and washed in PBS at 400g for 10min. Pelleted cells were then fixed in ice-cold 70% ethanol. Fixed cells were subsequently washed twice in PBS (600g; 10min) and incubated in 100 µl of ribonuclease (100 µg/ml; Sigma Aldrich) for 5 min (room temperature) prior to addition of 400 µl of propidium iodide (50 µg/ml; Sigma Aldrich) and 30 minutes incubation (37°C). Cells were then analysed for cell cycle status on FACScan flow cytometer (Becton Dickinson, USA) and using WinMDI and Cylchred softwares.

### 5. *RT-PCR*

35

Total RNA was extracted from cultured human cells using the Rneasy mini kit (Quiagen) and following the manufacturer's instructions. 3 µg of RNA was used in subsequent reverse transcription reactions. The RNA was

mixed with a poly T<sub>15</sub> oligo to 5 microgrammes/ml and heated to 75°C for 5 minutes. After cooling on ice, the following additional reagents were added; dNTPs to 0.4mM, RNase OUT (Promega) to 1.6 U/ $\mu$ l, Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLRvT) 5 point mutant (Promega) to 8 U/ $\mu$ l and the appropriate buffer (supplied by the manufacturer) to x1 concentration. The mixture was incubated for one hour at 37°C, heated to 70°C for two minutes and cooled on 10 ice.

PCR reactions were all performed in a total volume of 40 $\mu$ l. For each we used 1  $\mu$ l of the M-MLRvT reaction (as described above), 0.2nmols of each primer and 20 15 microliters of Redimix pre-mixed PCR components (Sigma). All reactions were cycled at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 60 seconds. 30 cycles were used for all primer sets except those for beta-actin, for which 23 cycles were used.

20 Primer sequences for RT-PCR:

AC133(U) : 5' CAG TCT GAC CAG CGT GAA AA 3'

AC133(D) : 5' GGC CAT CCA AAT CTG TCC TA 3'

25 Beta-actin(U) : 5' ATG TAC CCT GGC ATT GCC GAC 3'

Beat-actin(D) : 5' GAC TCG TCA TAC TCC TGC TTG 3'

CD34(U) : 5' TGA AGC CTA GCC TGT CAC CT 3'

30 CD34(D) : 5' CGC ACA GCT GGA GGT CTT AT 3'

CD38(U) : 5' GGG TGA TAC ATG GTG GAA GAG 3'

CD38(D) : 5' TGT GCA AGA TGA ATC CTC AGG 3'

35 HOXA9(U) : 5' AAT AAC CCA GCA GCC AAC TG 3'

HOXA9(D) : 5' ATT TTC ATC CTG CGG TTC TG 3'

HOXB4 (U) : 5' AGC GAT TAC CTA CCC AGC GAC 3'

HOXB4 (D) : 5' AGG GTC CCG GCA GGC CGC 3'

HOXB8 (U) : 5' TGG AGC TGG AGA AGG AGT TC 3'

5 HOXB8 (D) : 5' CGC TCC AGC TTC TGT TTC TC 3'

### Results

10 The results of the above described experiments are shown  
in Figures 1 to 6. Figure 1 shows the effect of HXP  
peptide on the cell growth of various cells and shows  
that the peptide protects immature blood stem cells  
(AC133<sup>+</sup> cells) by maintaining them in a quiescent state  
and slowing their growth. HXP inhibits the leukaemic  
15 cell lines (KG1a, KG1, HL60 and U937) rapidly (between 2  
and 6 hours), but the inhibition also appeared over  
longer time periods (Figure 3). (Whilst leukaemic cell  
lines can spontaneously proliferate in liquid culture  
systems, AC133<sup>+</sup> cells are primary haematopoietic  
20 stem/progenitor cells from umbilical cord blood. AC133<sup>+</sup>  
cells were therefore cultured with optimized cytokine  
cocktail necessary for their expansion which is  
reflected in their greater increase in proliferation  
relative to the leukaemic cell lines.) It appears that  
25 immature leukaemic cells such as KG1 and KG1a may be  
propelled into the cell cycle by the HXP peptide (Figure  
2), whereas the cell cycle of more mature leukaemic  
cells (HL60 and U937) is slowed.

30 Markers of stem/progenitor cells (AC133, CD34, HOXB4,  
HOXB8 and HOXA9) were diminished in KG1a cells treated  
with HXP peptide for 7 days, whereas markers of  
maturation/differentiation (CD38) were elevated (Figure  
4).

35

The effect of HXP peptide in maintaining stem cells in a  
quiescent state was found to be reversible. It will be

noted from Example 5 that withdrawal of the HXP peptide led to a resumption in growth of AC133<sup>+</sup> cells (Figure 5) and a return of markers of stem cells/progenitors (Figure 6).

5

Figures 6a) and b) show that HXP peptide slowed AC133<sup>+</sup> proliferation and this coincided with up-regulation of CD38 gene expression, whilst HOX B4, B8 and A9 genes were down-regulated from as early as day 3. On withdrawal of HXP peptide at 7 days (Figure 6c)), AC133<sup>+</sup> cells started to proliferate again, and reverted to near control levels when co-stimulated with HOX B4. This increase in proliferation correlated with progressive CD38 gene expression down-regulation and up-regulation of HOX B4, B8 and A9 genes.

10

15

#### Example 2: Cross-reactivity of HXP peptide with all PBX proteins

20

#### Methods

HXP and control peptides were as described in Example 1. KG1a cells were grown as described in Example 1. KG1a cells were cultured for 24 hours without treatment, or in the presence of 1 $\mu$ M HXP4 or control peptide. The cells were harvested and lysed. One aliquot of the cells was subject to cross-linking. Frozen cells were lysed using standard techniques and 100 $\mu$ l of lysate was incubated for 30 minutes at room temperature in 4mM 1-ethyl-3-[3-(dimethyl-amino)propyl]carbodiimide (EDC), 4mM sulpho-NHS, 20 mM HEPES (pH 7.5), 5 mM MgCl<sub>2</sub>, and 0.03% (w/v)  $\beta$ -DM to cross-link non-covalently associated proteins. The reaction was stopped by the addition of ammonium acetate to a final concentration of 50 mM. The other aliquot was frozen without cross-linking. Proteins were extracted from the cells of both aliquots by standard techniques and separated by gel

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30

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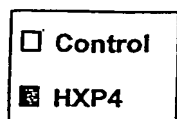
electrophoresis. The gels were probed by Western blot with an antibody raised against PBX1, 2 and 3 (sc-888, Santa Cruz Inc. USA) or an anti-beta actin antibody (sc-1615, Santa Cruz Inc. USA).

5

### Results

10 It will be noted from Figure 7 that whilst the PBX proteins from untreated and control peptide treated cells were associated with other proteins (ie. HOX proteins), none of the PBX isoforms from the HXP4 treated cells were associated with other proteins. This illustrates that the peptide of the invention has a global effect on all PBX proteins and thus prevents the  
15 interaction between all PBX and HOX proteins.





## Short-term effect of HXP on Cell Growth

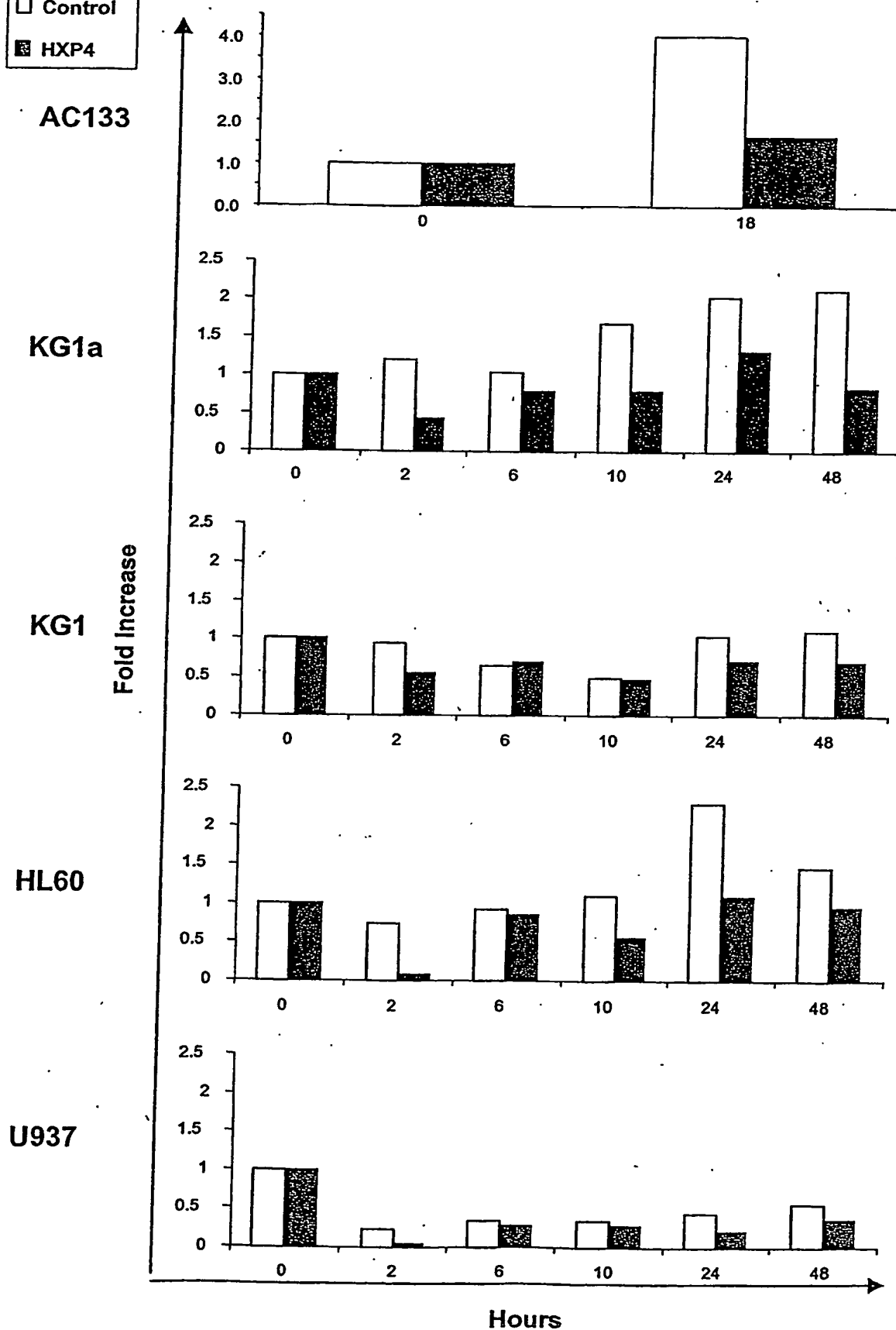
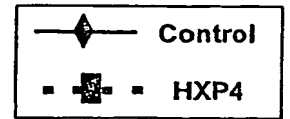


FIGURE 1

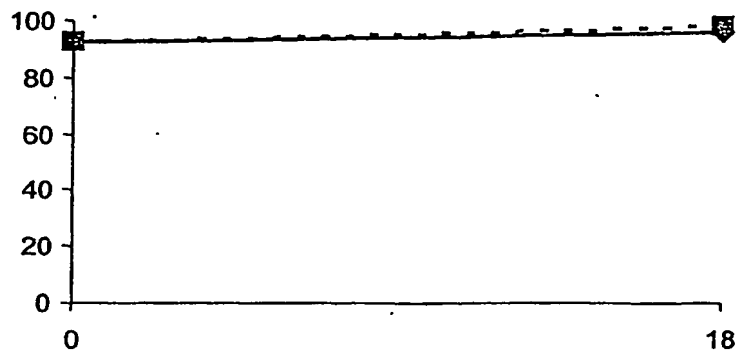


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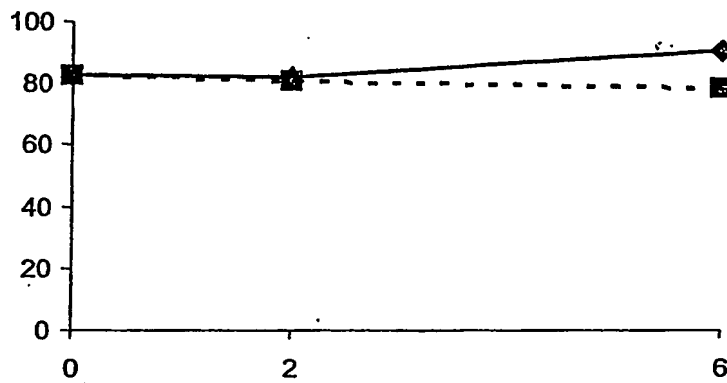
# Short-term effect of HXP on Cell Cycle



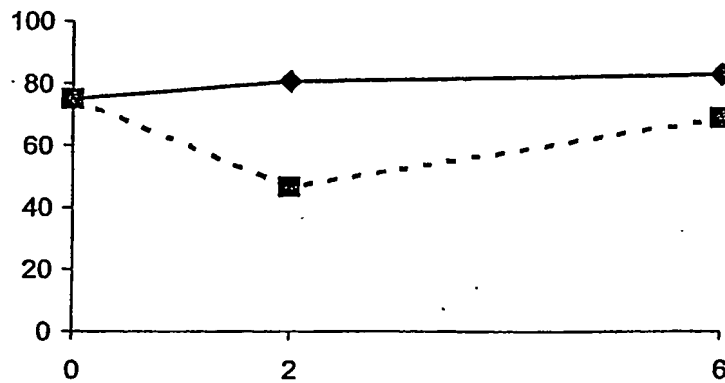
AC133



KG1a



KG1



U937

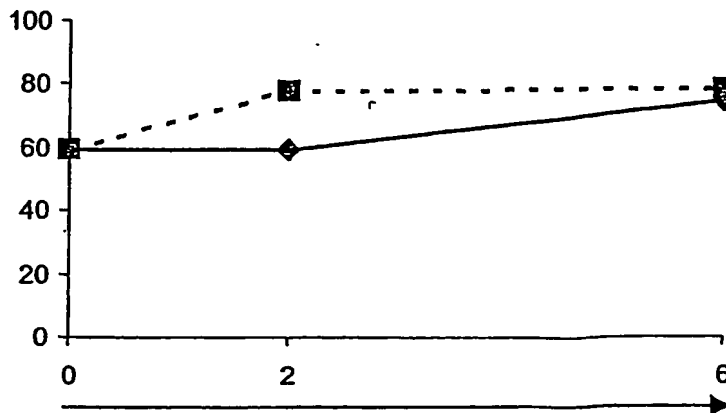


Figure 2

Hours

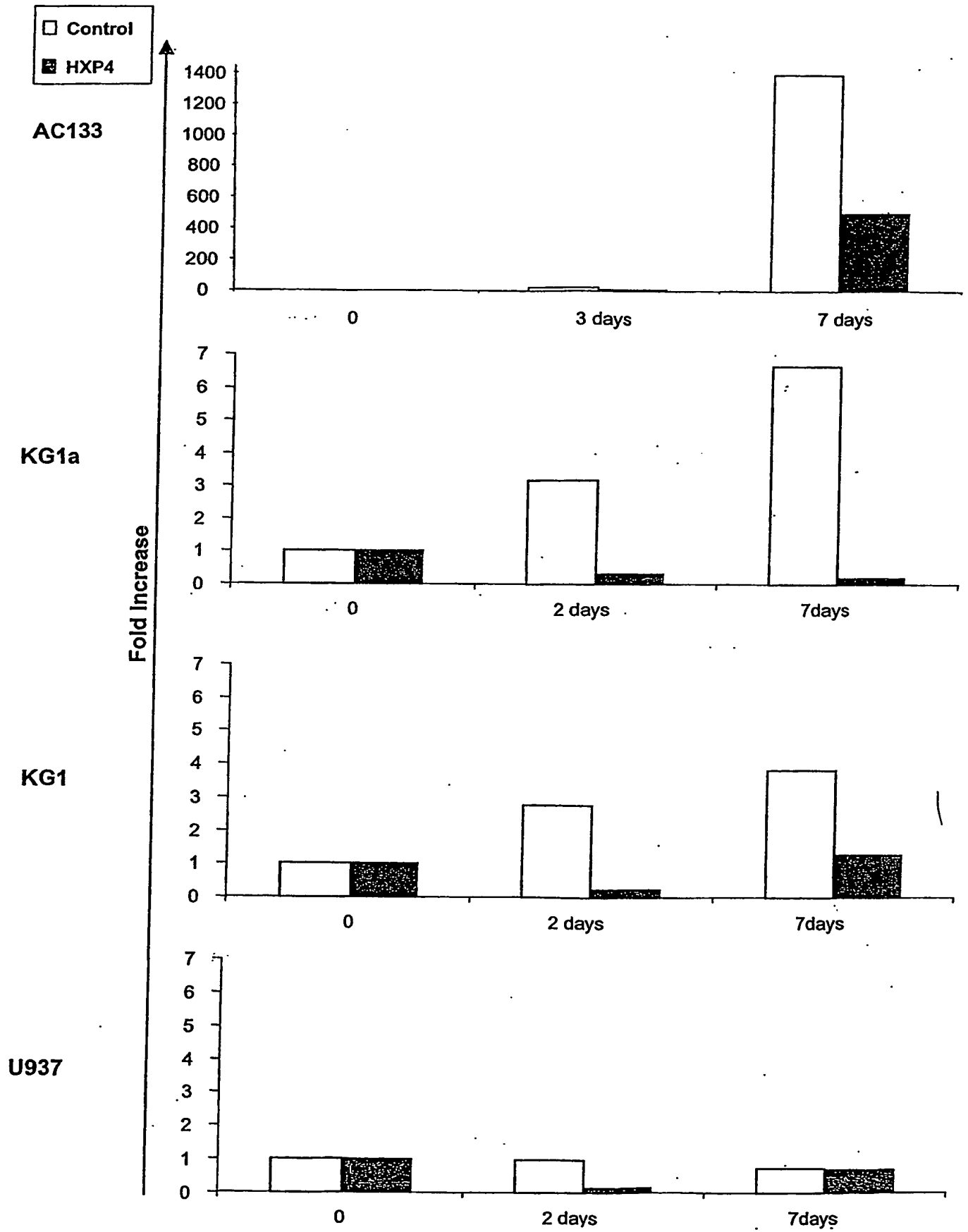


Figure 3

418

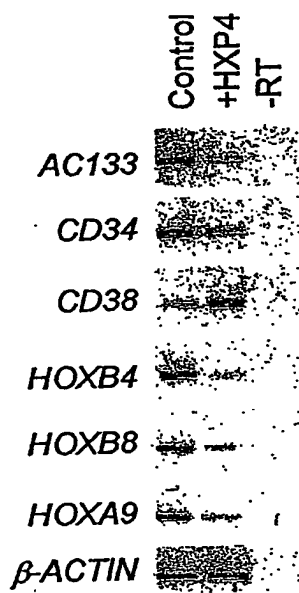


Figure 4

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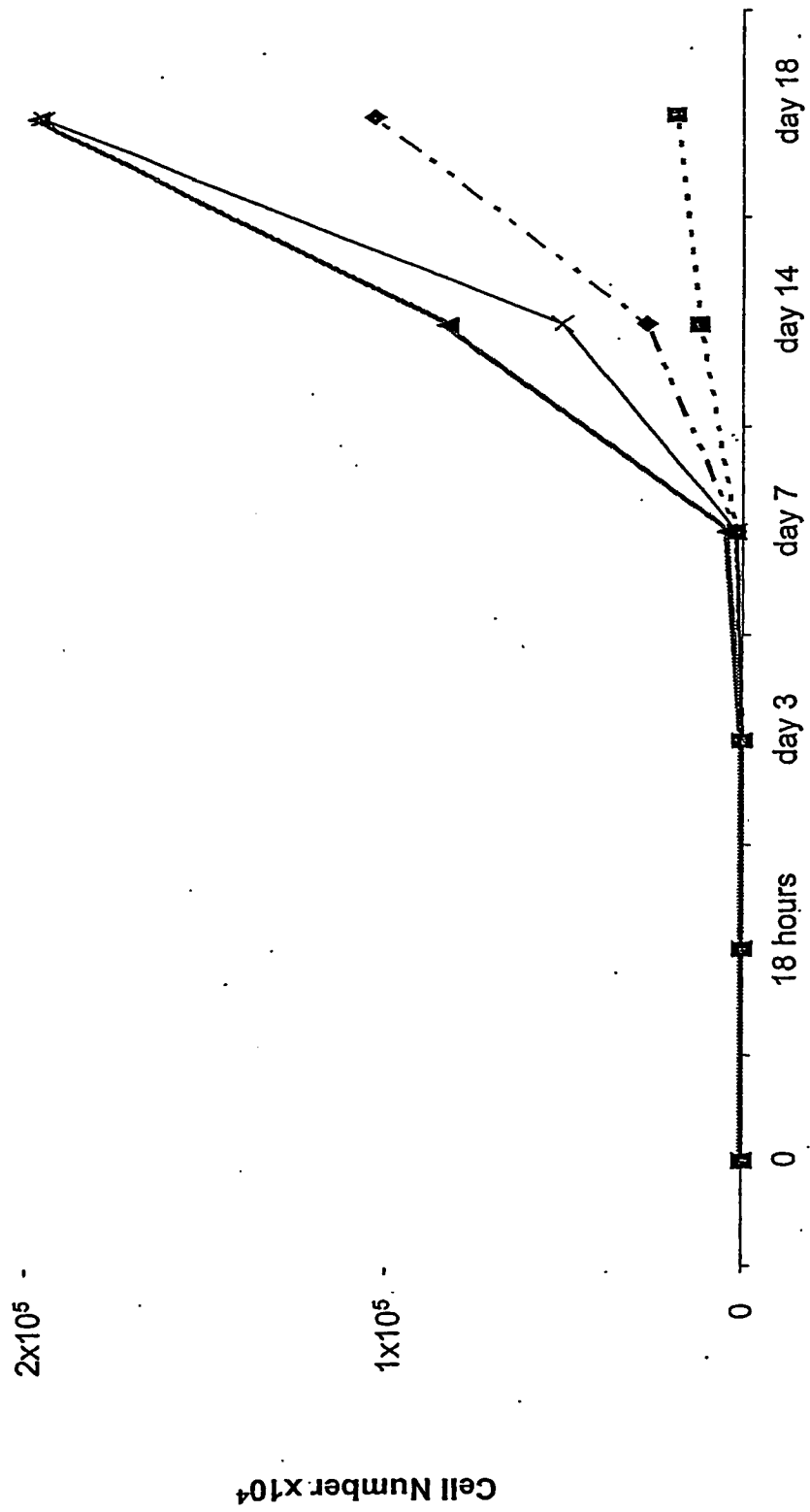


FIGURE 5

(a)

AC133

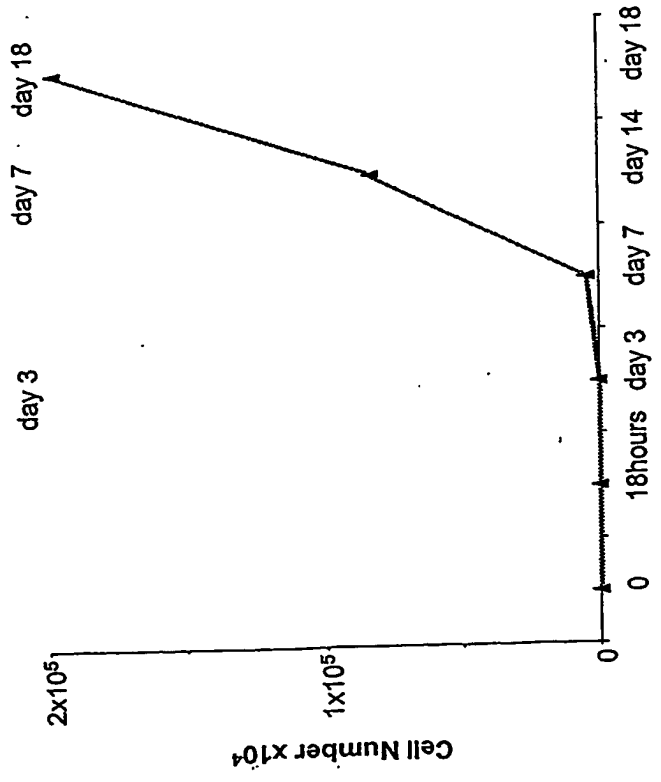
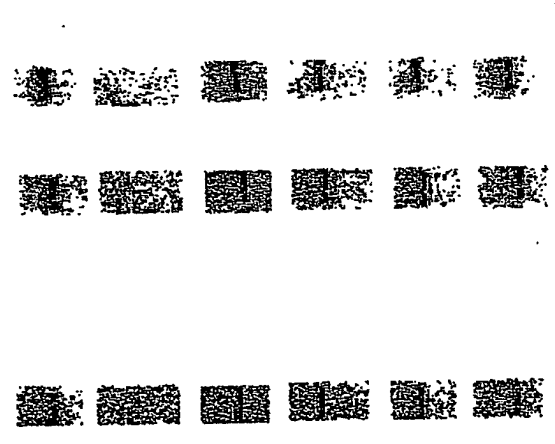
CD38

HOXB4

HOXB8

HOXA9

$\beta$ -actin



(b)

AC133

CD38

HOXB4

HOXB8

HOXA9

$\beta$ -actin

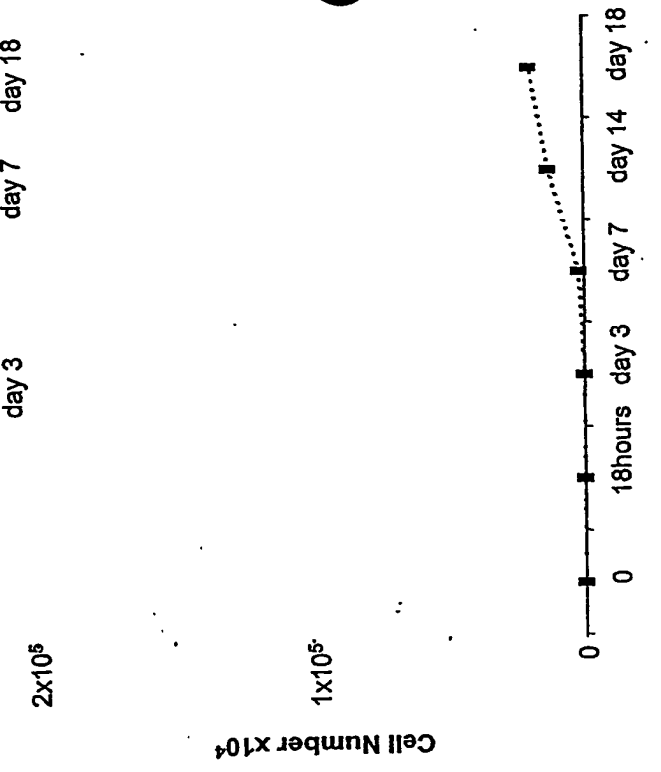
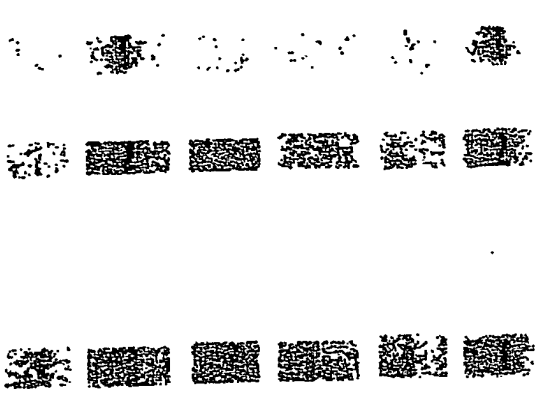
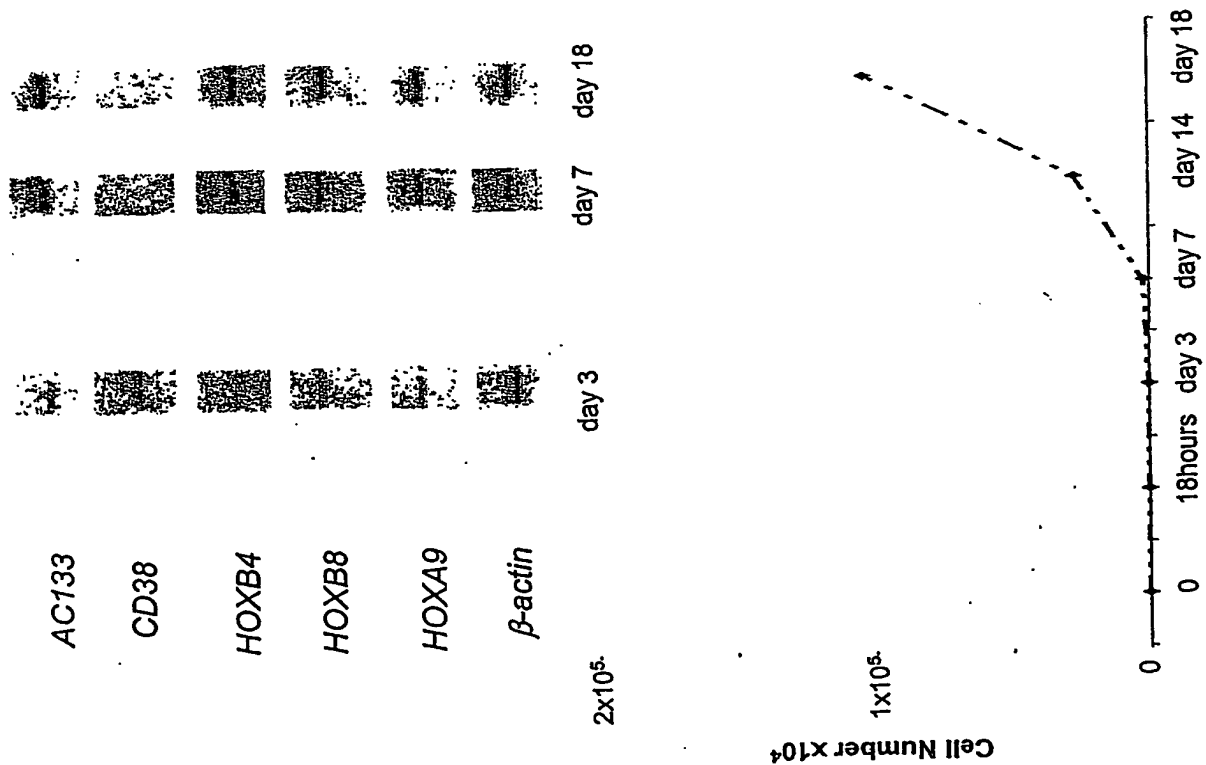


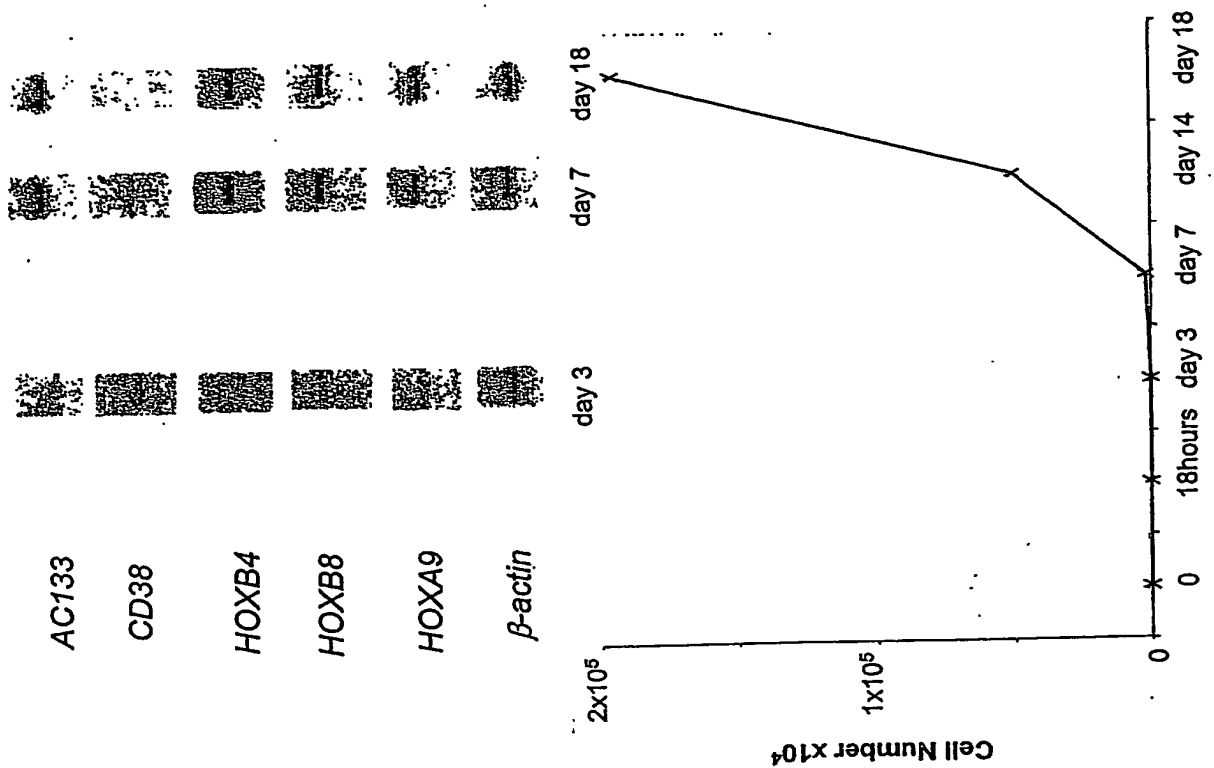
FIGURE 6a,b

Figure 6c,d

(c)



(d)



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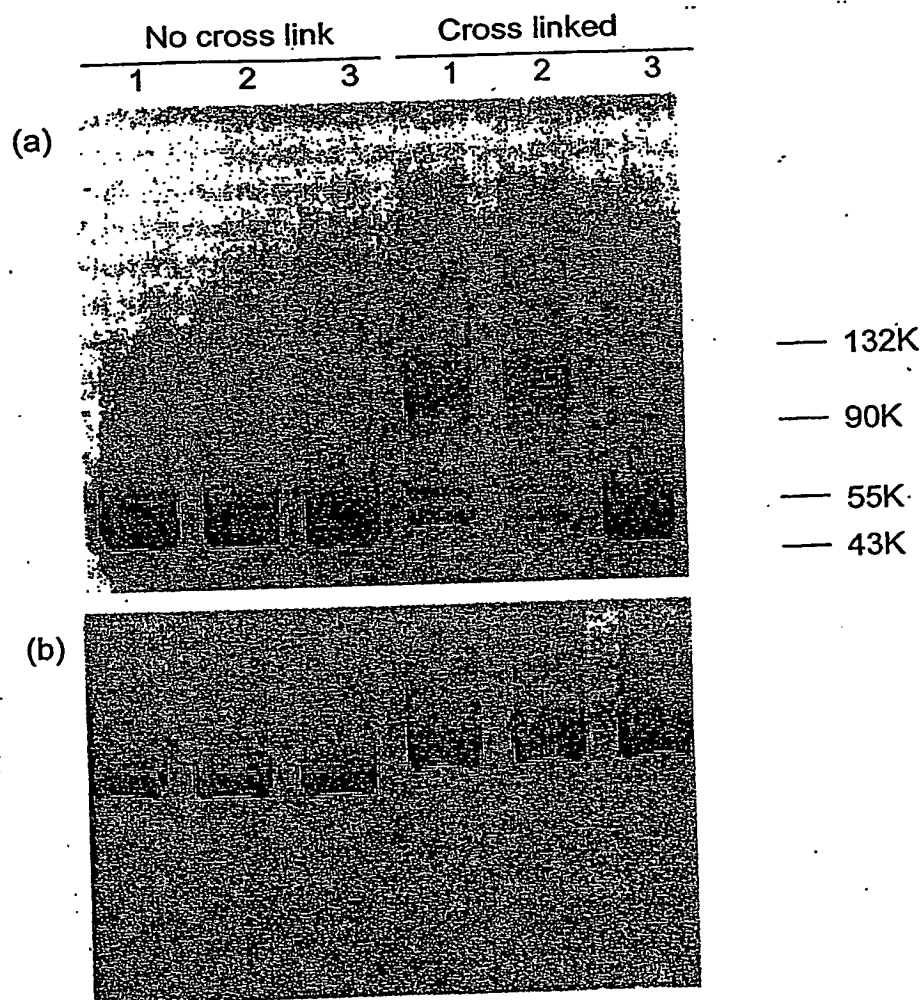


Figure 7

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